

FDA's Contributions to Advancing New Technologies for Developing Safe and Effective Influenza Vaccines

The infrastructure of FDA's scientific and regulatory processes contributes greatly to the availability of millions of doses of seasonal influenza vaccine every year, and thereby, contributes to influenza pandemic preparedness for the future. FDA's Center for Biologics Evaluation and Research's (CBER's) expertise, including the areas of research, vaccine manufacturing, and regulatory science, continue to facilitate the availability of safe and effective vaccines for the United States. For the past several years, CBER has been working to anticipate and address challenges associated with using new technologies in the development and licensure of vaccines to prevent influenza disease. Evaluation of new influenza vaccine candidates is based on existing knowledge and experience with regard to currently licensed influenza vaccines, as well as state-of-the-art science. A comprehensive, multi-faceted approach to these challenges is utilized by the Agency. These include:

- Conducting cutting-edge biomedical research in FDA laboratories pertaining to influenza vaccine development: Our research facilitates the development and evaluation of influenza vaccines with an emphasis on expediting influenza vaccine production and accelerating the public health response to emerging pandemic influenza virus strains. FDA scientists apply up-to-date scientific concepts from their research to regulation of vaccines.
- Routinely convening and participating in public workshops on emerging scientific and regulatory issues, where we share our knowledge of the latest science and seek input from other scientific leaders. In addition, convening public meetings such as our Vaccines and Related Biological Products Advisory Committee brings together a panel of outside experts from various scientific disciplines to assist the Agency in analyzing detailed scientific data and to provide recommendations on issues of public health significance.
- Issuing guidance documents to convey regulatory requirements and recommendations and to provide a scientific framework for the development of influenza vaccines. These documents facilitate regulatory and scientific exchange with industry to discuss regulatory pathways to licensure of influenza vaccine candidates produced using new technologies.

Background information and highlights of these key activities are provided below.

Why are prevention measures for influenza important?

Influenza is a contagious respiratory disease that is caused by a number of different influenza viruses. It can cause various symptoms, and may include fever, cough, sore throat, headache, body aches and chills and tiredness, and the disease can range from mild to deadly. Influenza disease reoccurs every year, generally in the late fall through early spring in the United States. Influenza seasons are unpredictable and can be severe. Over a period of 30 years, between 1976 and 2006, estimates of seasonal influenza-associated deaths range from a low of about 3,000 to a high of about 49,000 people.

Because influenza viruses can change, a new virus can emerge suddenly and spread around the world. This is called an influenza pandemic and most people will not have immunity against the new virus. The impact or severity tends to be higher in pandemics than for seasonal influenza in part because of the much larger number of people in the population who lack pre-existing immunity to the new virus. When a large portion of the population is infected, even if the proportion of those infected that go on to develop severe disease is small, the total number of severe cases can be quite large.

During 2009, a new and very different influenza virus designated as 2009 H1N1 spread worldwide causing the first pandemic in more than 40 years. It is estimated that the 2009 H1N1 pandemic resulted in more than 12,000 influenza-related deaths in the U.S. Unlike seasonal influenza, nearly 90 percent of the deaths occurred among people younger than 65 years of age. It also caused many more cases of viral pneumonia than is normally seen with seasonal influenza.

What is the most effective method to prevent influenza?

Vaccination remains the cornerstone of preventing influenza; this is true for both seasonal and pandemic viruses. Currently, all influenza vaccines licensed in the United States are derived from viruses grown in chicken eggs. Egg-derived influenza vaccines have a long and successful track record of safety and efficacy in the United States. Manufacturers of influenza vaccines have traditionally relied on embryonated hens' eggs to grow these viruses due to the ease of production in eggs. The infrastructure for egg production is rather simple and straightforward, and the regulatory pathway to licensure, as well as analytical assays, are well established. Since influenza vaccines were first introduced in the 1940s, the basic manufacturing process for influenza vaccines using hens' eggs has changed little. Currently, there are six vaccine manufacturers, licensed by FDA to produce influenza vaccine for the U.S., all of which are egg-based. According to the World Health Organization, billions of doses of inactivated influenza vaccine have been produced to date using egg-based manufacturing.

While the current method is tried and true and rests on a strong scientific foundation and a tremendous amount of experience, having to rely on only one method to manufacture influenza vaccines is not ideal. Therefore, CBER has issued regulatory guidance and is engaging in scientific efforts to describe additional options available for the manufacturing of these important vaccines.

Potential alternatives to egg-based manufacturing for influenza vaccines are under development. These include cell culture technology, recombinant technology, and adjuvant technology. Each of these technologies, along with other CBER efforts, is described in more detail below.

Cell Culture Technology

Cell culture technology is used in the manufacture of other vaccines and work is underway to make this a viable option for influenza vaccines. Cell-based technology involves a production process similar to egg-based technology. As with egg-based technology, the vaccine antigen (the active substance of the vaccine that stimulates a protective immune response) is produced from

the influenza virus. However, rather than using fertilized eggs as the medium for producing the influenza vaccine, cell-based technology typically uses cells grown in bioreactors (sealed tanks) suspended in a suitable growth medium. The cells infected with the influenza virus are used for the production of the vaccine. The cells are derived from mammalian cells which have been established as continuous cell line banks that are extensively tested and well characterized.

The use of cell culture for the production of influenza vaccines has some advantages over the traditional egg-based vaccines, including potential higher virus yields for many strains of influenza, the potential for more rapid scale-up of vaccine manufacture, and the ability to bank and characterize the cells used in vaccine production. On the other hand, not all virus strains grow better in cell culture (e.g., the isolates of the pandemic H1N1 2009), and it will be important to address potential amplification in cell culture of adventitious agents co-isolated with the original influenza virus during vaccine development. Once the influenza virus is propagated and harvested, the processes for purification, filling and formulation for vaccines using cell cultures likely would be similar to egg-based methodologies. Of note, the critical and rate limiting step remains the availability of a seed virus that expresses growth characteristics resulting in virus yields that would enable a cell-culture production process sufficient to provide vaccine quantities for pandemic outbreaks.

CBER Influenza Vaccine Research Activities – Cell culture technology

CBER has developed a chemical induction strategy to investigate the presence of latent viruses (adventitious agents) for evaluating the safety of novel cell substrates that are needed for the development of influenza vaccines using new technologies. CBER is currently assessing the application of emerging broad virus detection methods such as massively parallel or deep sequencing, virus microarrays, and long-range PCR with mass spectrometry (PLEX-ID) for detection of known and unknown viruses from chemically-induced cell substrates for vaccine safety. The results from these studies will facilitate development of influenza vaccines in novel cell substrates.

CBER is developing methods to investigate the origin and nature of reverse transcriptase (RT) activity in novel cell substrates that might be used for manufacture of influenza vaccines using new technologies. RT activity is generally associated with retroviruses, therefore, CBER's work in this area will help to assess the safety of these new cell substrates.

Adventitious agents are historically detected by a battery of *in vitro* and *in vivo* tests; however, CBER has developed new, sophisticated molecular technologies that are being applied to the detection of adventitious agents.

The issue as to whether DNA in vaccines and its effect on safety concern has been debated for nearly 50 years without resolution; CBER scientists are: first, establishing quantitative assays to determine whether the levels of residual DNA in vaccines represent a safety concern; and second, determining how this concern can be mitigated.

Relevant Guidance Document

In February 2010 CBER released the Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications. This guidance is intended to aid manufacturers in developing safe and effective cell-based viral vaccines. It will help manufacturers who wish to use new cell substrates for the production of vaccines, providing advice to manufacturers about the scientific principles of cell substrate development and describing tests that may be used to evaluate cell substrates intended for use in viral vaccine production.

Meetings and Workshops

In September 2008, CBER convened its VRBPAC to engage in scientific discussion regarding the suitability of Madin-Darby Canine Kidney Cells, or MDCK cells, rather than eggs for the manufacture of live attenuated influenza virus vaccine or LAIV. Detailed information concerning this meeting can be found at Vaccines and Related Biological Products Advisory Committee Meeting – September 25, 2008 – Transcripts – Part 1 Part 2 Addendum.

Prior to the 2008 meeting, CBER also convened its VRBPAC in November 2005 to consider the use of MDCK cells for the manufacture of inactivated influenza vaccines. While some lines of MDCK cells are not tumorigenic, others are highly tumorigenic. Thus, one goal of this meeting was for the Committee to comment on CBER's approach to risk assessment of highly tumorigenic cell substrates. Information on this meeting can be found at <http://www.fda.gov/ohrms/dockets/ac/cber05.html#VaccinesandRelatedBiological>.

In September 1999, CBER-sponsored an international workshop (*Evolving Scientific and Regulatory Perspectives on Cell Substrates for Vaccine Development*) that took place in Rockville, MD. The proceedings were published in *Development in Biologicals*, volume 106, 2001.

Recombinant Technology

Recombinant technology utilizes a host cell to express a specific antigenic protein, rather than the use of a live virus. Typically bacteria, yeast, or mammalian cells are used as the host. This manufacturing process would also employ large production tanks, and has the potential to increase both the capacity and speed of production.

CBER Influenza Vaccine Research Activities – Recombinant technology

Using recombinant DNA technology, CBER is developing a panel of pre-pandemic influenza hemagglutinin (HA) antigens based on the vaccine candidates recommended by the World Health Organization. These HA antigens will be utilized for preparation of necessary potency reagents and for analyzing their antigenicity in animal models.

CBER researchers are developing and evaluating new recombinant methods to produce influenza HA and NA including bacterial and mammalian expression systems. Recombinant influenza proteins expressed in bacteria and virus-like particles expressed in mammalian cells are properly

folded and immunogenic in animal models. Development of such methods has the potential to provide reagents and tools for vaccine characterization as well as for the evaluation of protective immune responses following vaccination.

For the 2009 H1N1 pandemic, CBER scientists used reverse genetics to develop candidate vaccine strains that were suitable for vaccine production and were provided to manufacturers.

Relevant Guidance Documents

In 2007, CBER released two guidances for industry; one specific to seasonal influenza vaccines and one specific to pandemic influenza vaccines. These guidances address the clinical data needed to demonstrate the safety and effectiveness of influenza vaccines. The guidance documents are applicable to the development of vaccines using new technologies, including recombinant technology.

Meetings and Workshops

In late 2009, CBER convened its VRBPAC to discuss a Biologics License Application for a seasonal influenza vaccine, Purified Recombinant Influenza Hemagglutinin. The product discussed was a seasonal influenza vaccine, produced in an insect cell expression system. Details and the outcome of the meeting are available on the web.

In July 2011, CBER participated with the World Health Organization and representatives from other countries in a workshop to discuss methods for generation of candidate influenza vaccine viruses. These candidate vaccine viruses are provided to vaccine manufacturers to enable seasonal vaccine production. This production is normally accomplished by a classical reassortment process in eggs. However, for highly pathogenic strains of influenza virus, this process may not work. In such cases, the process of reverse genetics has to be used to make the candidate vaccine viruses. Influenza vaccine candidates produced by using reverse genetics method are patented and fall under intellectual property right (IPR). Scientists from across the world, including CBER, are convening to investigate the potential usefulness of methods not covered by existing IPR.

Adjuvant Technology

Adjuvants have been used in a number of vaccines against other bacterial and viral pathogens, and are being investigated for use in influenza vaccines. The purpose of formulating vaccines with adjuvants is to increase the immune response to the vaccine, which allows a decrease in antigen dose, or a greater efficacy, or both.

CBER Research Activities – Adjuvant technology

CBER researchers conducted studies on oil-in-water adjuvant (MF59) when combined with pandemic influenza vaccines. They measured the strength of human antibodies against influenza vaccine in the presence of MF59, looking at cohorts of different age groups (toddlers, children,

and adults) vaccinated with the swine origin-H1N1 vaccine, and in a separate study, adults vaccinated with avian-H5N1 vaccine. In both studies, subjects received either unadjuvanted or MF59-adjuvanted inactivated subunit vaccines. The researchers showed that the adjuvant increased the magnitude, diversity, and most importantly, the binding avidity of the antibodies, potentially a crucial factor when antibodies in limiting quantities encounter virus. Specifically, antibodies—with the help of the MF59 adjuvant—are able to latch strongly onto receptor binding domains on the virus key surface protein (hemagglutinin) and prevent infection. These findings demonstrated for the first time that oil-in-water adjuvants can improve antibody quality in a manner that is predicted to improve protection against pathogenic influenza strains.

CBER researchers are conducting studies using cells that are able to detect potential toxicity of adjuvants in tissue culture. This provides important information on safety prior to studies in humans.

CBER scientists have created phage display libraries to fingerprint antibody responses to influenza vaccines that aid in evaluating the impact of an adjuvant. CBER is conducting these studies using various vaccine platforms, including, egg-based, virus-like particles, DNA Plasmid and plant-based.

Relevant Guidance

The guidance documents for industry described previously that address the clinical data needed to demonstrate the safety, immunogenicity, and efficacy of influenza vaccines, both pandemic and seasonal are applicable to the development of vaccines using adjuvanted technology.

Meetings and Workshops

In December 2008, CBER convened a workshop in partnership with the National Institute of Allergy and Infectious Diseases of NIH pertaining to adjuvants and adjuvanted vaccines. The workshop assessed the scientific knowledge base regarding vaccine adjuvants to facilitate the development of a research agenda to improve the safety and efficacy assessments of adjuvanted vaccines for the treatment and prevention of infectious diseases. Specific information on the workshop is available at

[Public Workshop: Adjuvants and Adjuvanted Preventative and Therapeutic Vaccines for Infectious Disease Indications – December 2-3, 2008.](#)

What else is CBER doing to facilitate development of new influenza vaccines?

CBER is responsible for producing, calibrating, and providing to the vaccine manufacturers and the global community the yearly reagents needed to assess potency of influenza vaccines, both seasonal and pandemic. Because the composition of vaccines to prevent seasonal influenza is evaluated annually and changed as necessary to protect against circulating influenza viruses, new potency-testing reagents must also be generated to accurately evaluate the vaccines. Timely preparation of potency reagents by CBER is a key step for influenza vaccine production, and it is

extremely important that additional approaches to reagent development be available, particularly in the event of an emerging pandemic influenza virus.

In addition, CBER has efforts underway to assess alternatives to the currently used potency assay for influenza vaccines, including new assays which may alleviate the need to produce large quantities of reagents for each new vaccine strain.. Currently, potency of all inactivated influenza vaccines is determined using a single radial immunodiffusion (SRID) assay. This assay requires the production of large quantities of specific reagents (reference antigen and reference antiserum) for each virus strain. Production of assay reagents requires approximately 2 months. New potency assays with improved accuracy and sensitivity would accelerate vaccine manufacture.

CBER Research Activities – Potency Reagents and Assays

CBER developed an alternative method for preparing strain-specific antibody that did not require the growth or purification of influenza virus, and was not limited by the success of the traditional technique of bromelain digestion and purification of virus hemagglutinin (HA). The results demonstrate a feasible approach for addressing one of the challenges in producing inactivated pandemic influenza vaccines--the timely production of one of the critical potency-testing reagents. This is especially important in the case of an influenza virus that emerges suddenly and starts to spread very rapidly, as the pandemic (H1N1) 2009 influenza virus did, thus necessitating the rapid production of a vaccine. The new strategy can also be used for seasonal influenza vaccines. Follow-up research by CBER has shown that this technique is applicable to other systems as well, including bacterial and mammalian systems.

CBER scientists are conducting research on promising assays that are being evaluated for development as improved influenza vaccine potency assays. The research has several components to ensure a comprehensive approach and includes 1) an ELISA assay using subtype specific monoclonal antibodies to bind and quantify conformationally correct HA, 2) an antibody-independent, label-free Mass Spectrometry method to quantify peptides representative of HA with native conformation in influenza vaccines, and 3) a surface plasmon resonance (SPR) based receptor binding assay to quantify the amount of properly folded HA trimers and oligomers in reference antigens and vaccines. Each assay will be compared to the current SRID assay for potency determination and their suitability to monitor vaccine stability.

Meetings and Workshops

A workshop, jointly organized by Health Canada, CBER, and the WHO, was held in Ottawa, Canada in July 2010 to evaluate lessons learned from potency testing of pandemic (H1N1) 2009 influenza vaccines and considerations for future potency tests.

The specific goals of the workshop were to:

- exchange knowledge and experience gained in vaccine release and immunization around the world throughout the 2009 influenza pandemic
- formulate plans to address gaps in our knowledge of the use of alternative approaches to assess potency of influenza vaccines

- identify possible ways to incorporate such assays into influenza vaccine regulation

As a first step in the outcomes, a recommendation was made for improvements to the SRID assay, which is the standard to measure potency of inactivated influenza vaccines. In parallel, alternative methods should continue to be evaluated for potential future application. This is underway, as described above.

CBER Research Activities – Universal Influenza Vaccine

Researchers in the Center for Biologics Evaluation and Research and the Centers for Disease Control and Prevention developed a "universal," off-the-shelf vaccine designed to reduce illness and slow the spread of disease caused by new influenza A viruses that emerge suddenly, spread quickly, and for which there is no specific vaccine available. A single dose of the vaccine reduced illness and virus levels in mice later infected with highly virulent (disease-causing) H1N1 and H3N2 (seasonal influenza), and H5N1 (bird flu). A link to a recent publication is posted [on the web](#).

The key to the new vaccine's broad protection is that it is designed to trigger immune responses against two protein targets in all influenza viruses that change over time much more slowly than the specific proteins targeted by traditional vaccines. The vaccine is administered nasally, which enables it to stimulate the immune system in the mucous membranes, where influenza viruses launch their infection.

This type of vaccine, if effective in humans, could be stockpiled and then used to reduce deaths and severe illness in the event of delayed production of traditional vaccine against a newly emergent influenza virus.

What does the future hold?

Influenza viruses are highly unpredictable, and constantly recombine with each other to form new influenza viruses. Which influenza viruses will circulate from year to year is also unpredictable, just as predicting when the next influenza pandemic will occur is equally unpredictable; however, there are certainties in the present to help us prepare for what the future may bring. Every year new influenza vaccines are made, and each year can present new challenges. Because influenza viruses mutate, each year's vaccine may be different from the preceding year. Therefore, one of the biggest challenges in the process is to produce a new safe and effective vaccine every year, and make it available for distribution to the public in a timely manner. There is no vaccine other than influenza where a new vaccine formulation must be made every year. Influenza vaccine contains three components which target the three main circulating strains of influenza (two influenza A viruses, and one influenza B virus) identified through worldwide surveillance to be the most common during the season. The intense activities of preparing for the seasonal influenza every year have provided CBER with considerable experience to handle the demands of an influenza pandemic. CBER is a key participant in building vaccine capacity and assisting the influenza vaccine enterprise to meet public health

needs. CBER facilitates vaccine development, speeds vaccine availability, and ensures vaccine quality and safety using the most modern scientific methods.